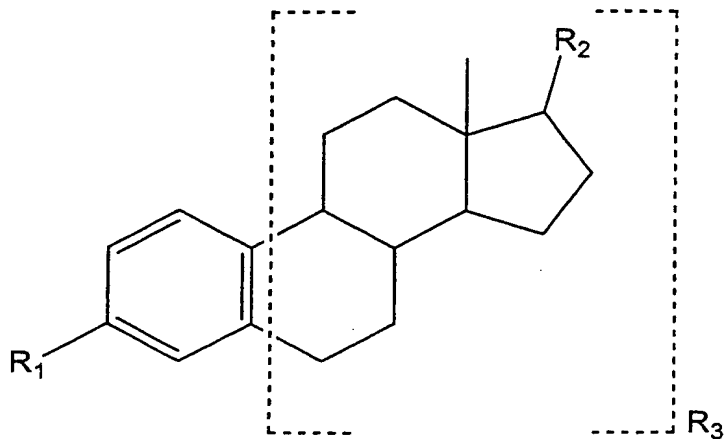




Appl'n No.: 10/829,028
Title: Method and Compositions for.....
Inventors: Stavros C. Manolagas *et al.*
Replacement Sheet

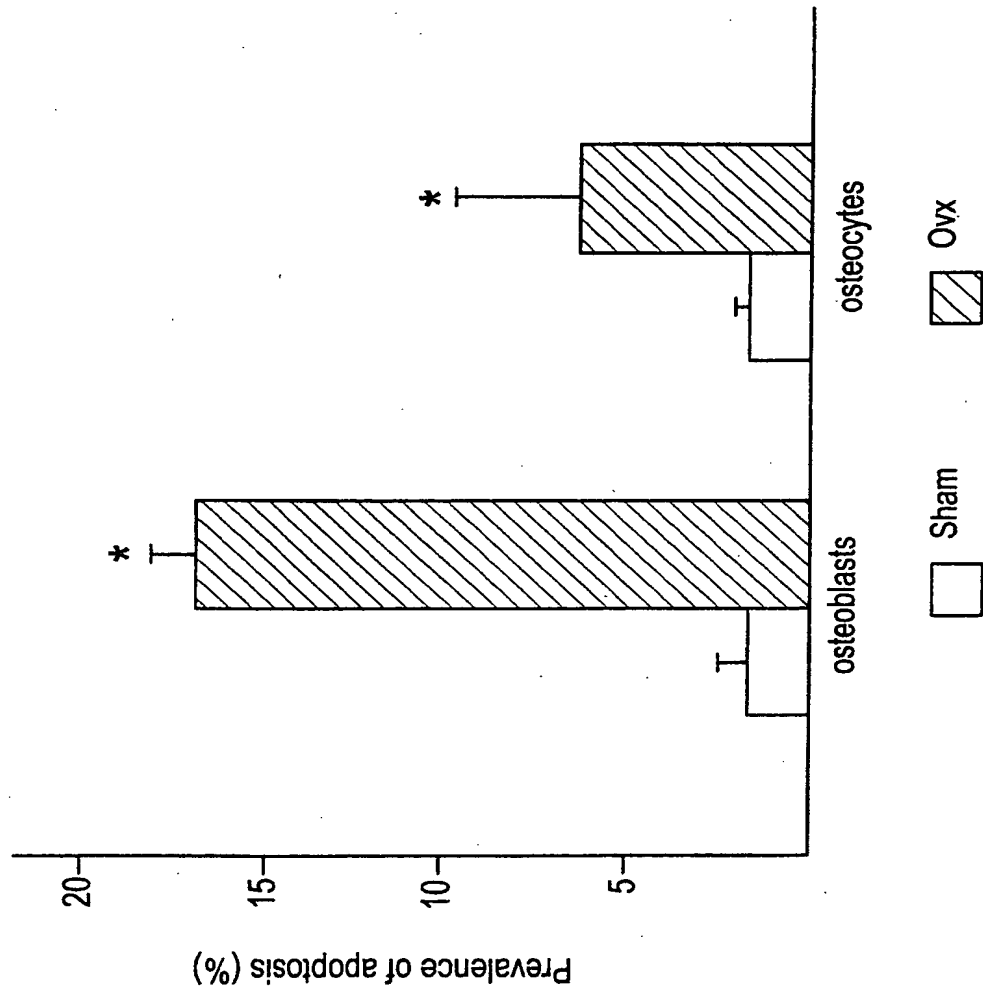
FIG. 1

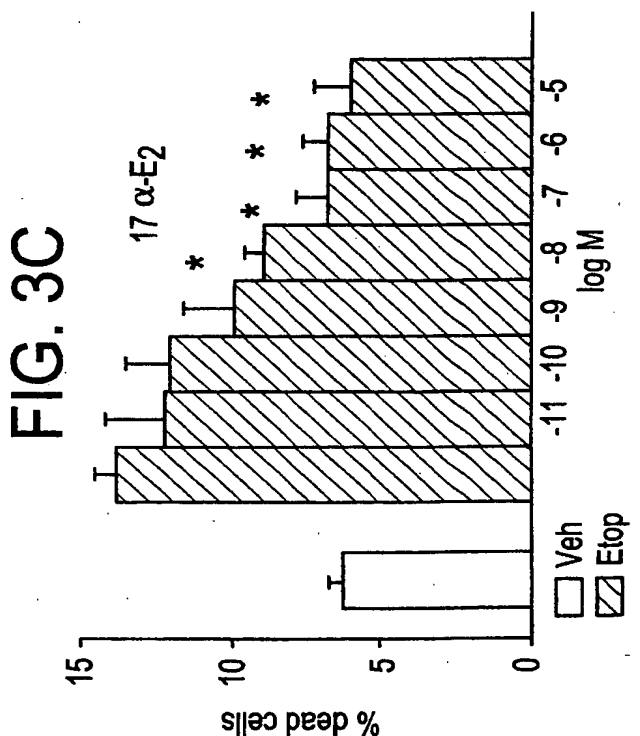
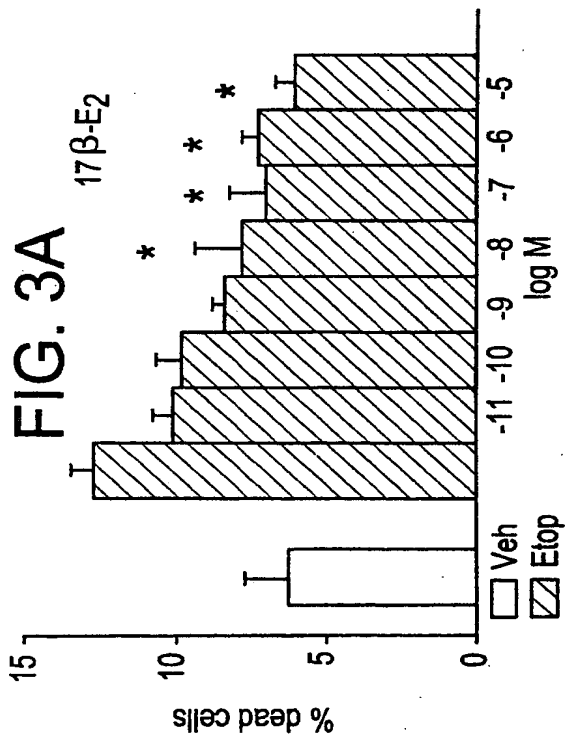
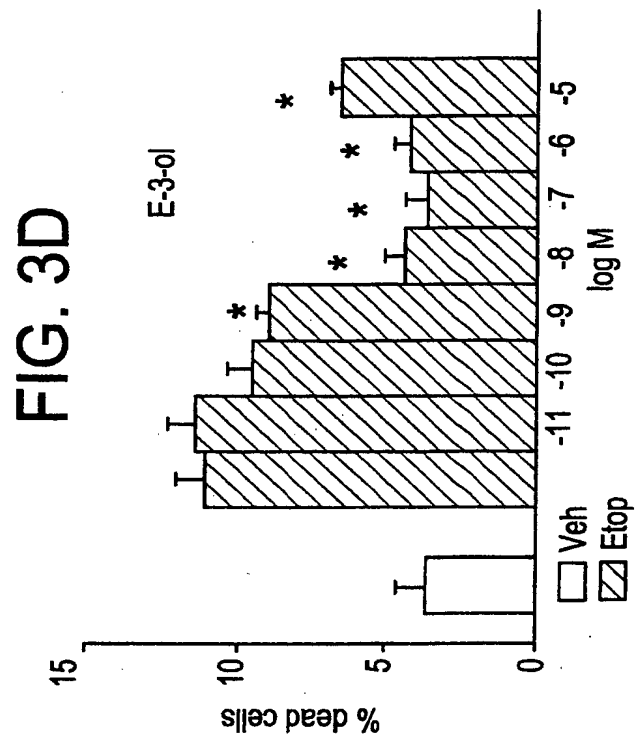
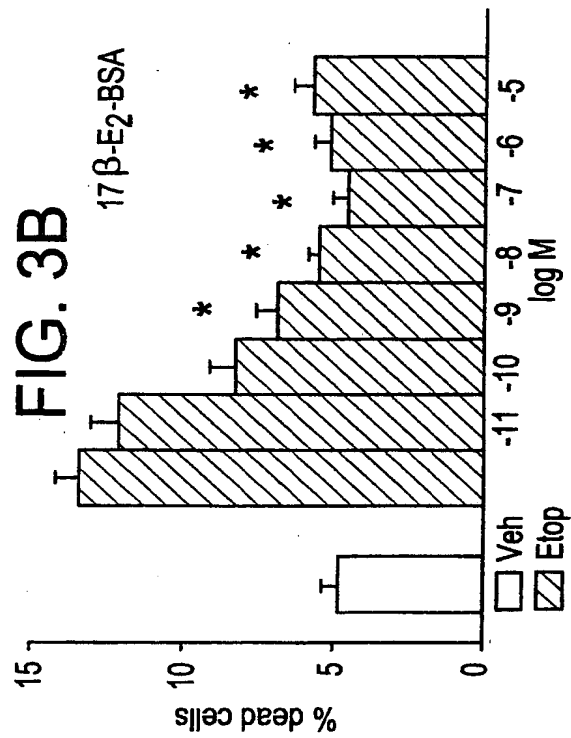


General structure of activators of non-genomic Estrogen-Like Signalling (ANGELS).

Estrogen deficiency causes increased apoptosis of osteoblasts and osteocytes in murine vertebral bone.

FIG. 2





Inhibition of apoptosis of osteoblastic cells.

FIG. 4A

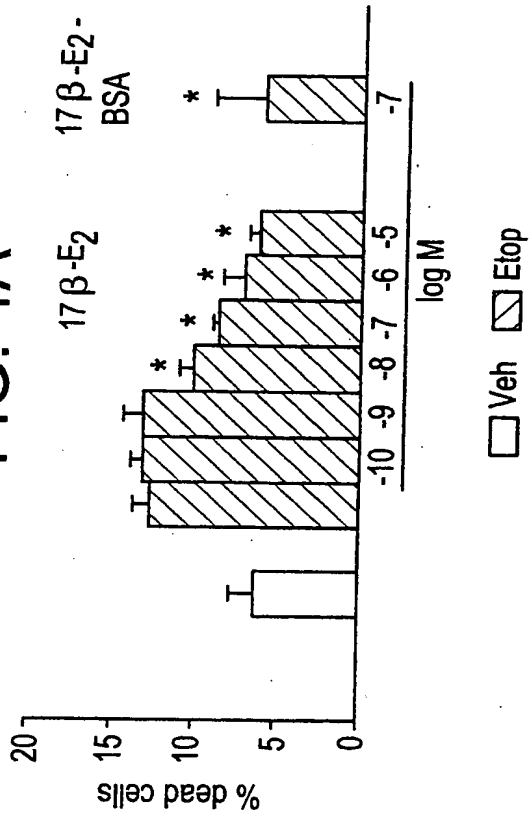


FIG. 4B

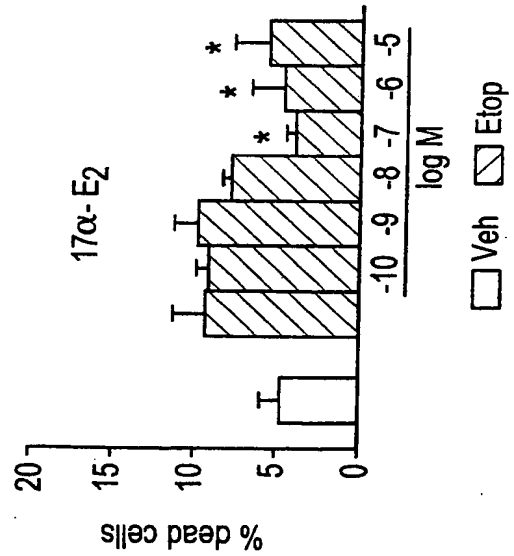
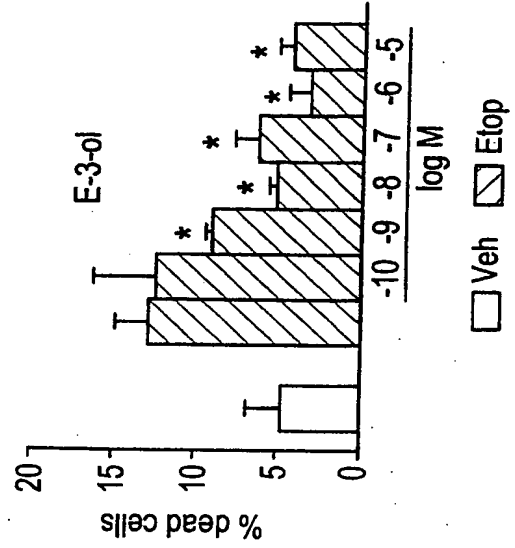
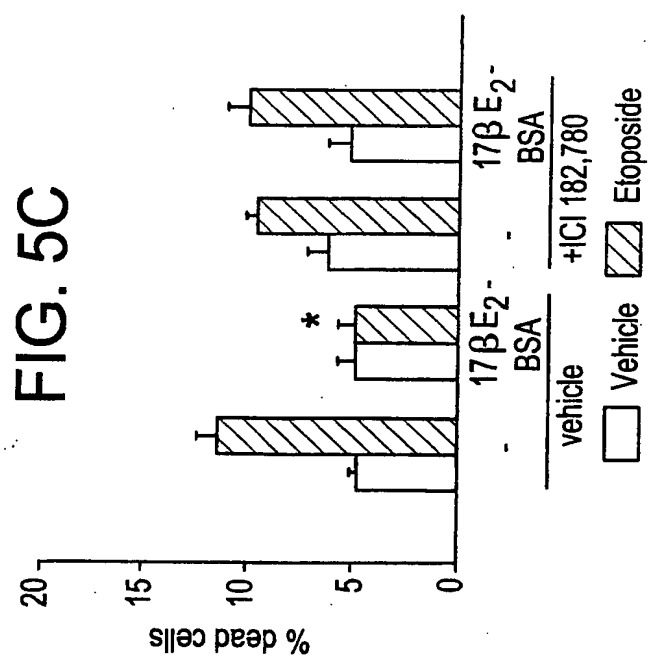
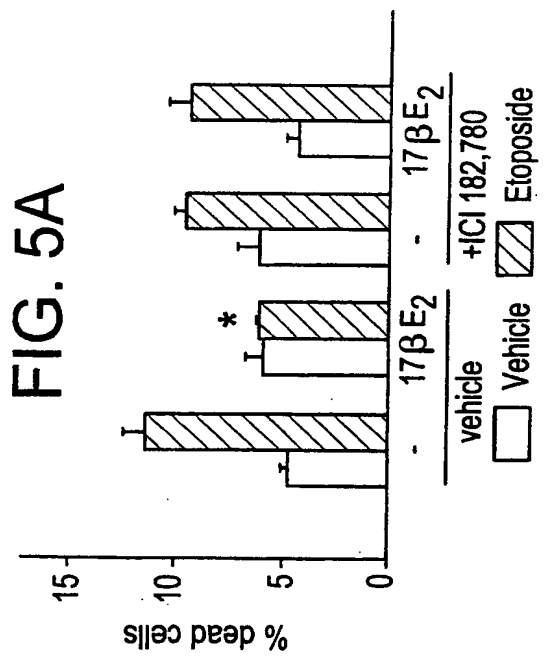
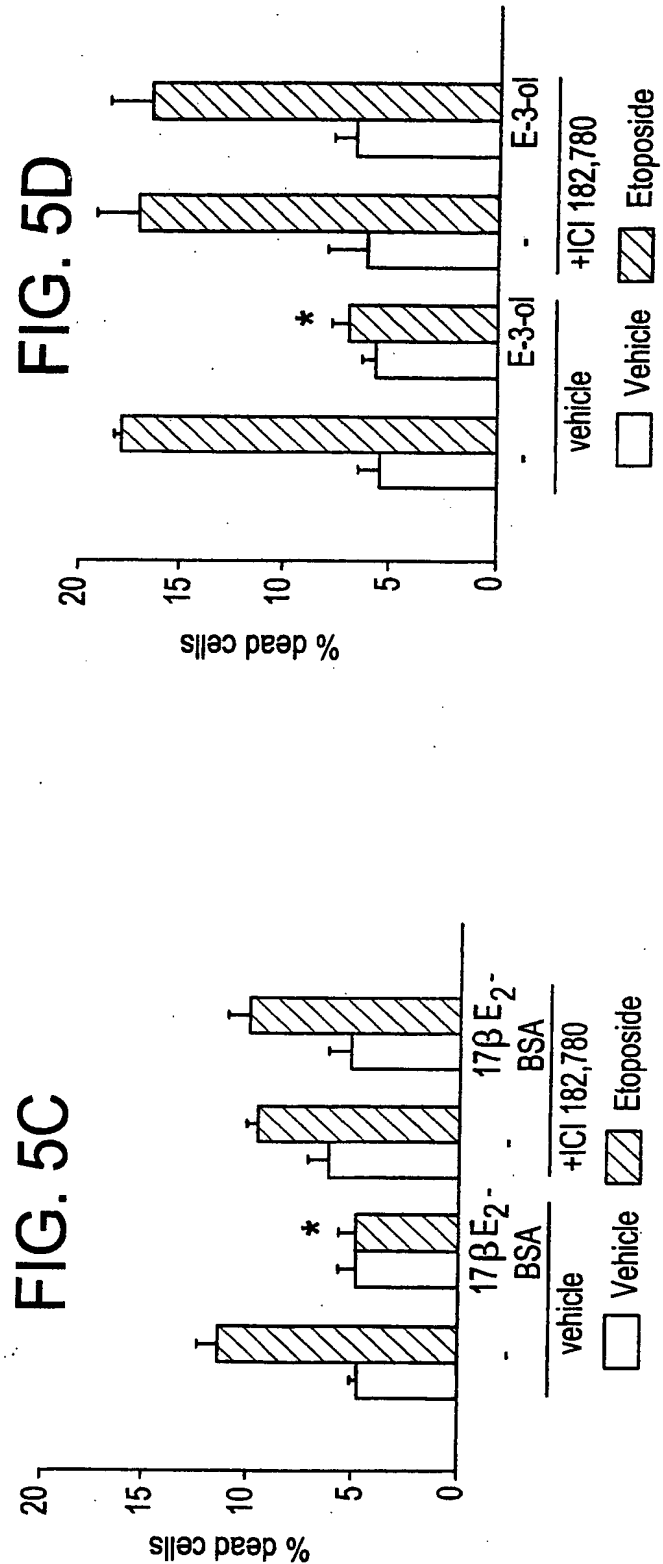
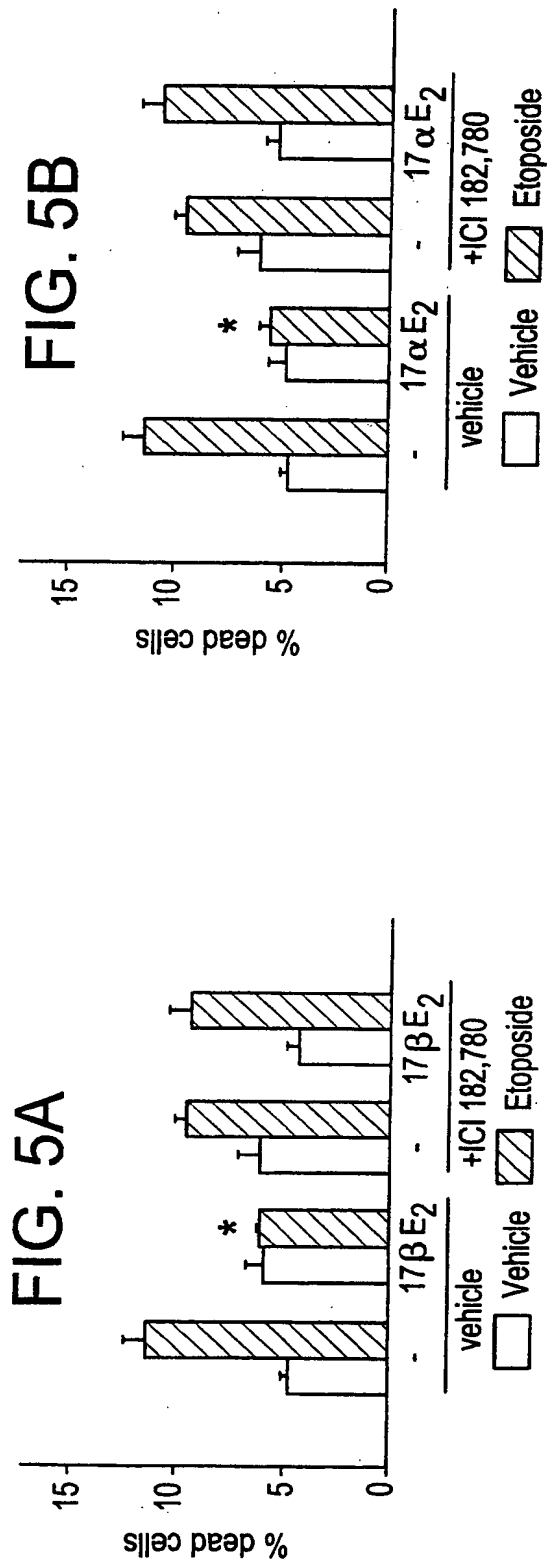


FIG. 4C



Inhibition of apoptosis of MLO-Y4 osteocytic cells by ANGELS



Blockade of the anti-apoptotic effect of estrogen and ANGELS by ICI 182,780 in osteoblastic cells

FIG. 6A

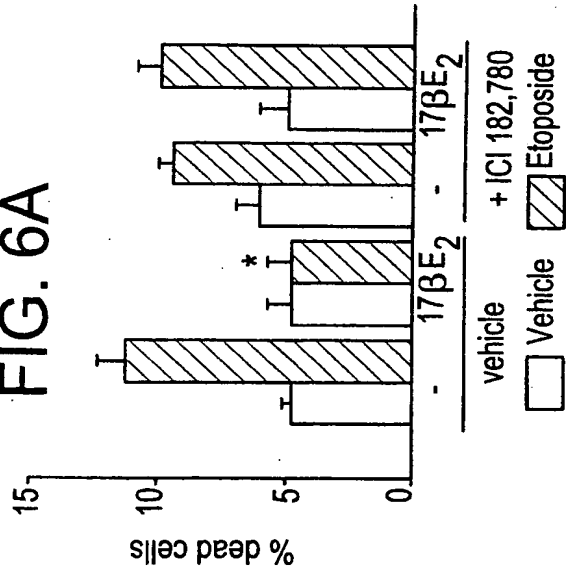


FIG. 6B

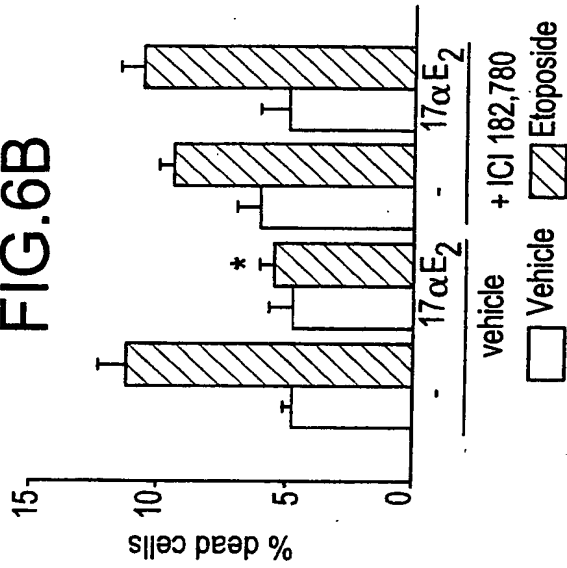


FIG. 6C

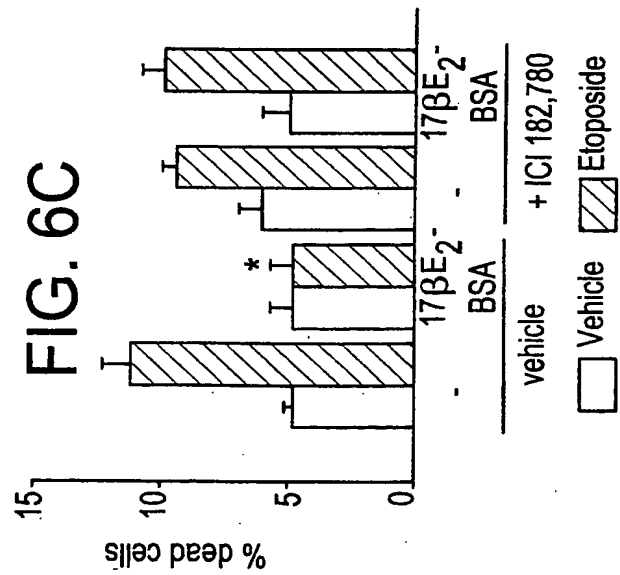
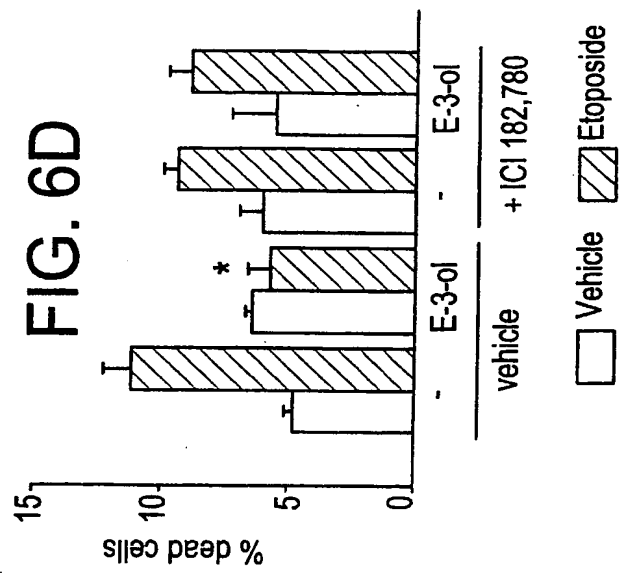
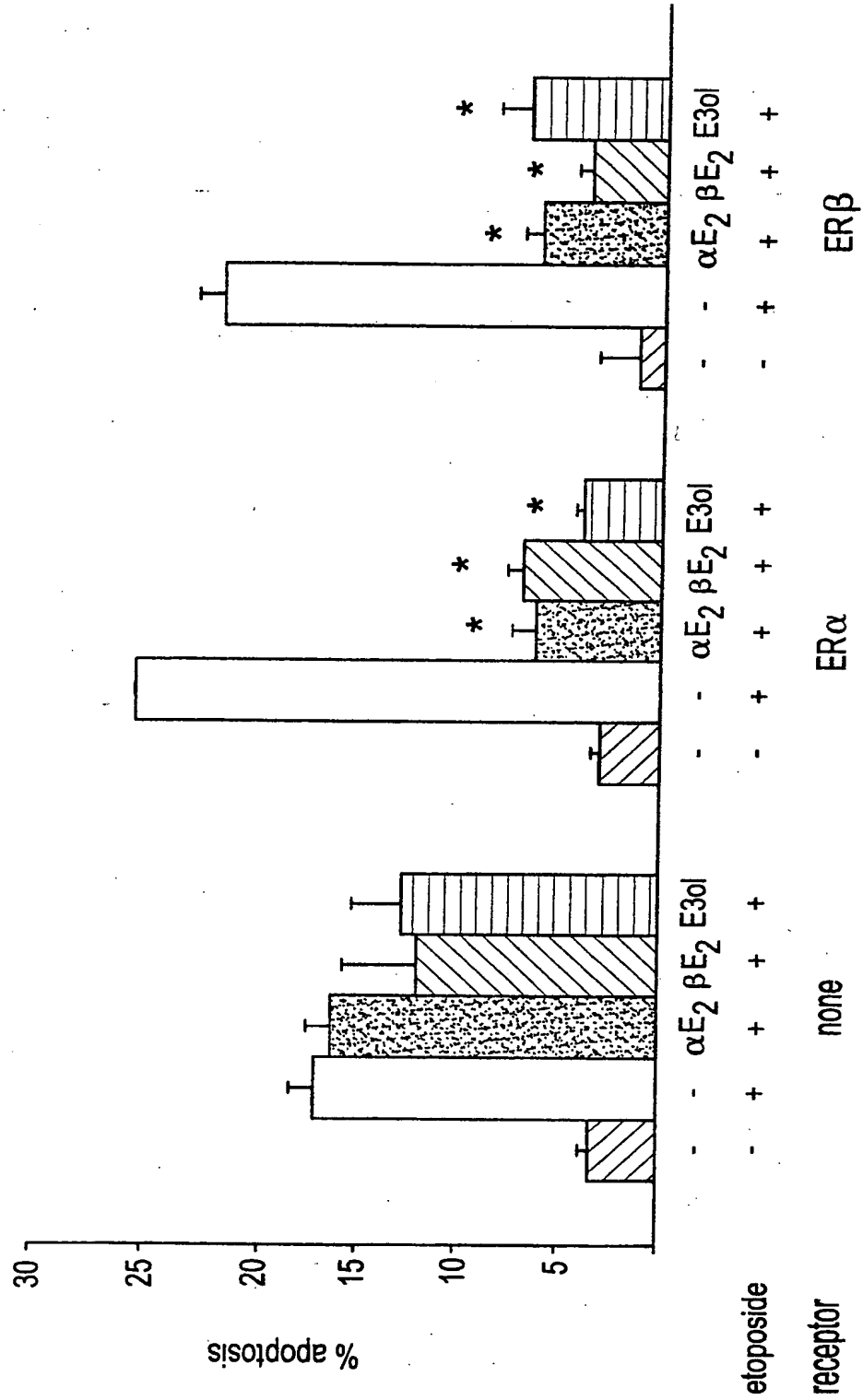


FIG. 6D



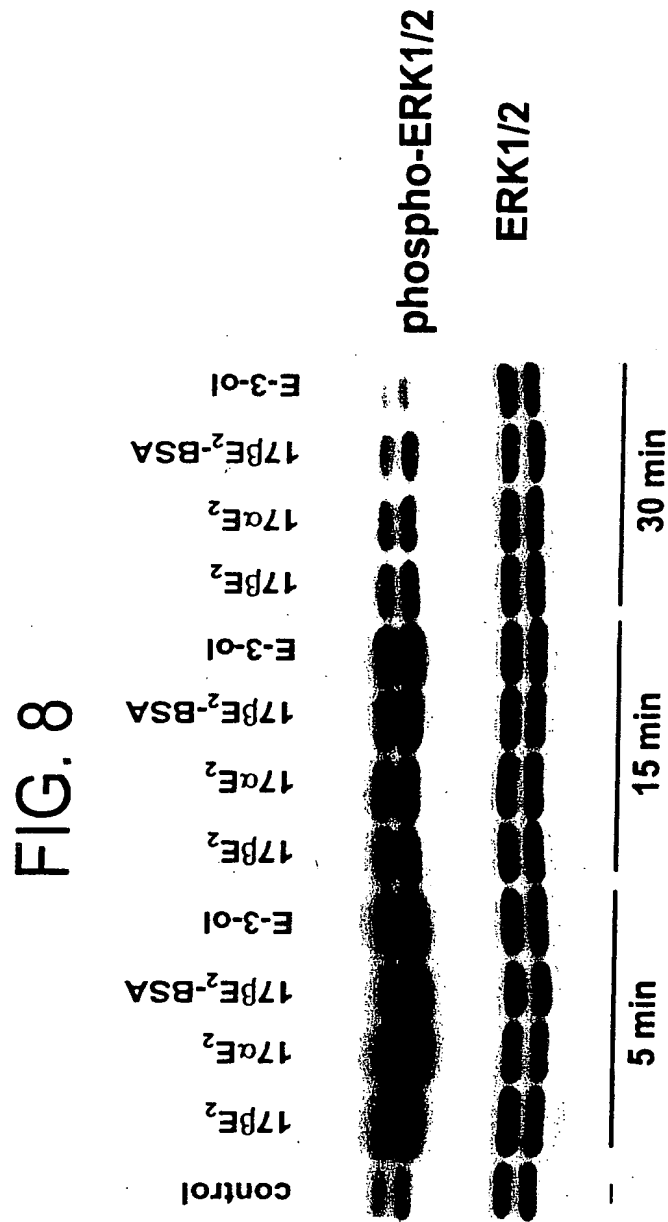
Inhibition of the antiapoptotic effect of estrogen and ANGELS by ICI 182,780 in MLO-Y4 osteocytic cells

FIG. 7



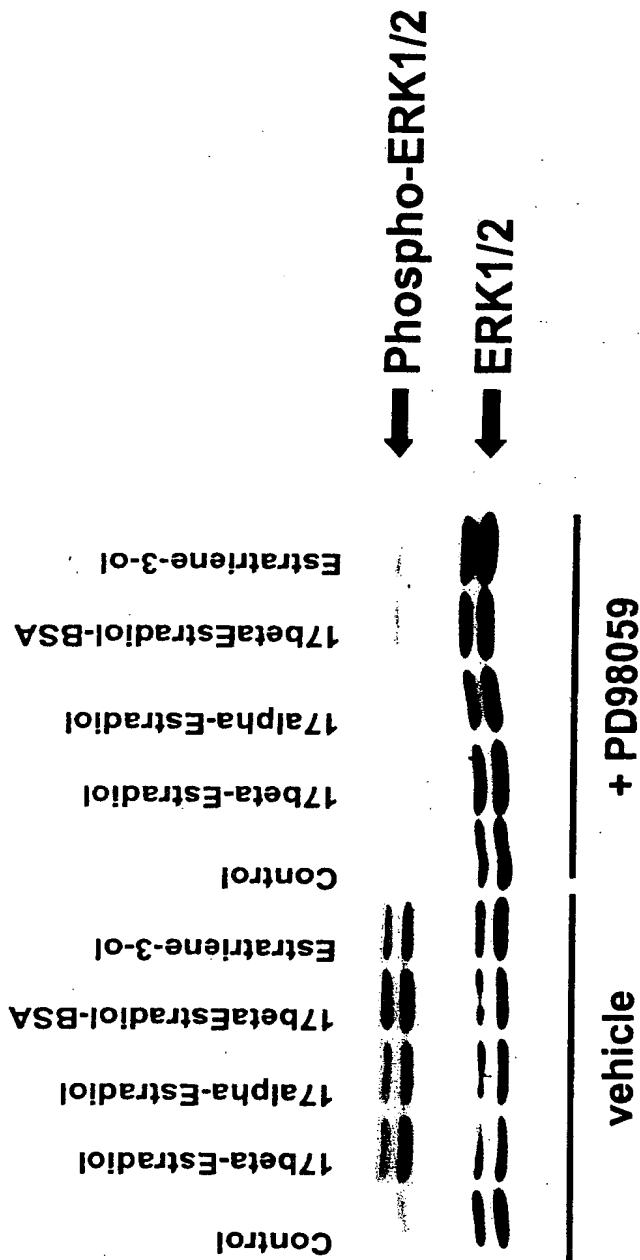
Estrogen receptor a or b is required for the antiapoptotic effects of 17b estradiol, 17a estradiol, and estratriene-3-ol on etoposide-induced apoptosis (experiment 1/21/99).

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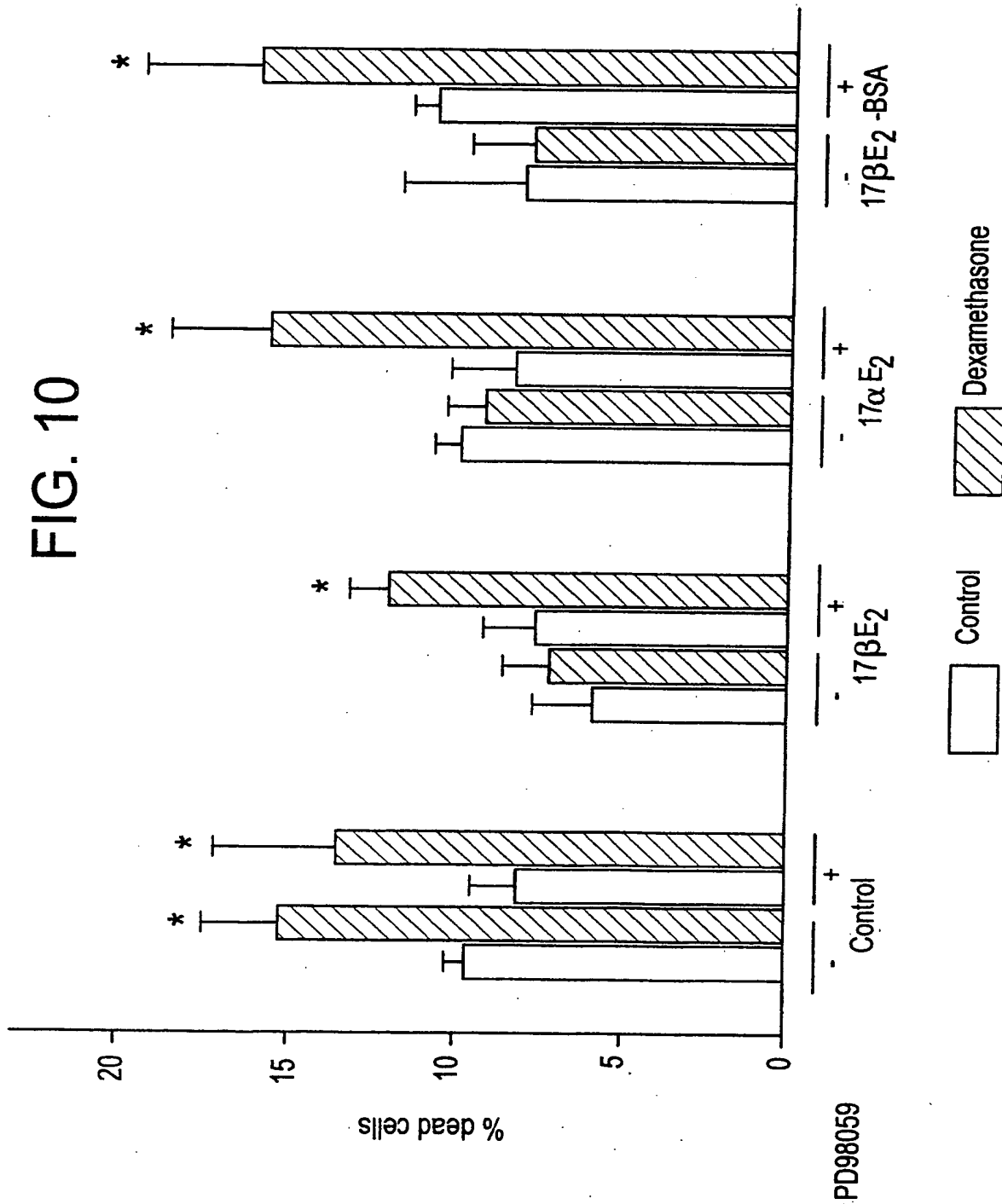
Activation of Extracellular Signal Regulated Kinases (ERKs)

FIG. 9

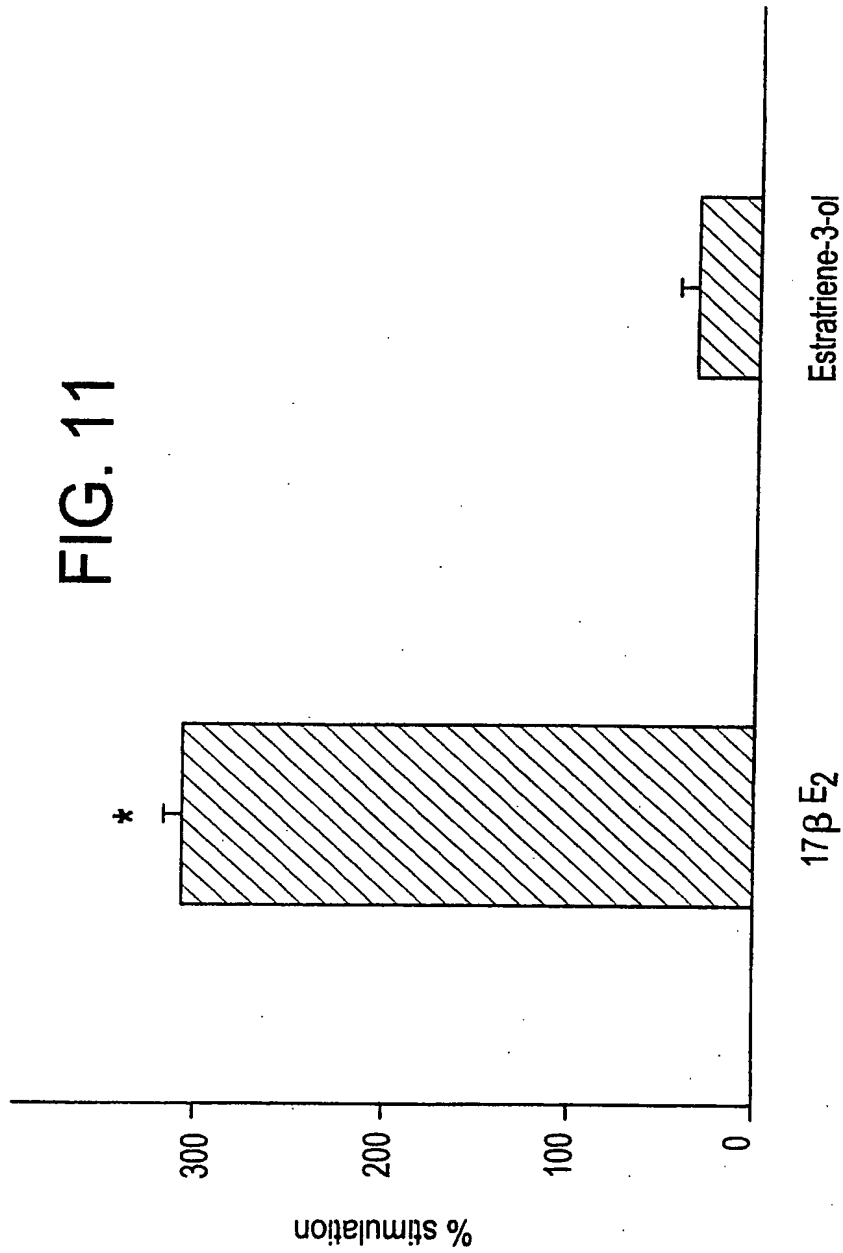


The effect of estrogenic compounds on the activation of ERK1/2 is blocked by a specific inhibitor.

FIG. 10

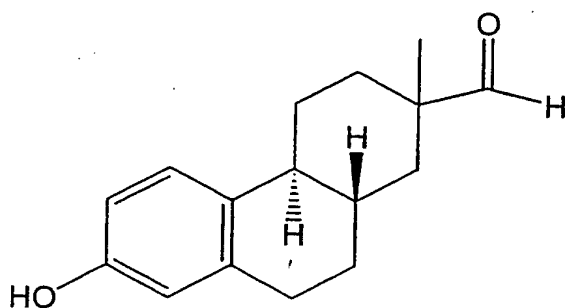


The specific inhibitor of ERK activation abolishes the anti-apoptotic effect of the estrogenic compounds.

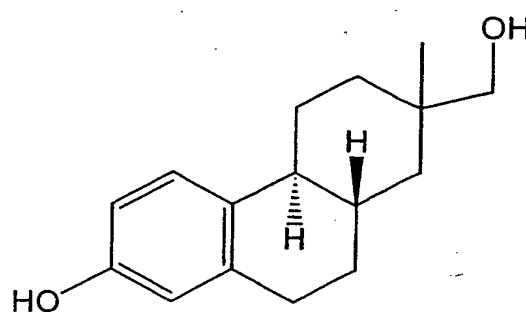


Unlike 17β estradiol, estratriene-3-ol does not transactivate an estrogen response element through ERα.

FIG. 12



$C_{16}H_{20}O_2$
MW=244
[2S-(2a, 4a α , 10a β)]-1, 2, 3, 4, 4a, 9, 10,
10a-Octahydro-7-hydroxy-2-methyl-2-
phenanthrenecarboxaldehyde



$C_{16}H_{22}O_2$
MW=246
[2S-(2a, 4a α , 10a β)]-1, 2, 3, 4, 4a, 9, 10,
10a-Octahydro-7-hydroxy-2-methyl-2-
phenanthrenemethanol

FIG. 13

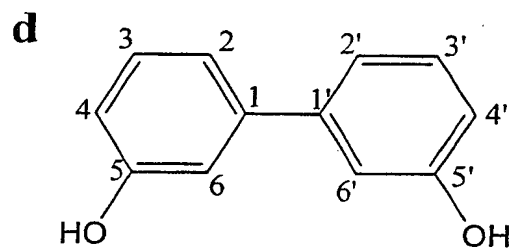
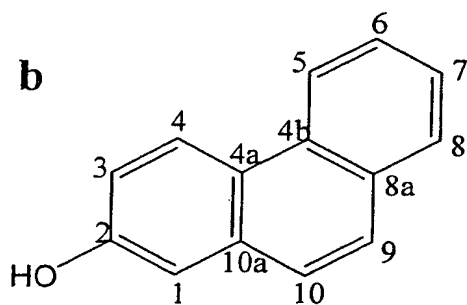
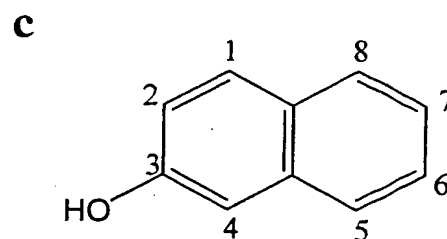
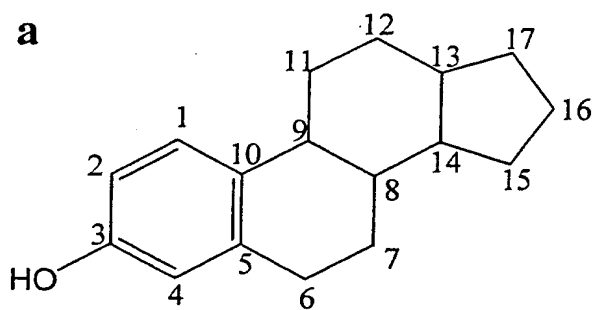
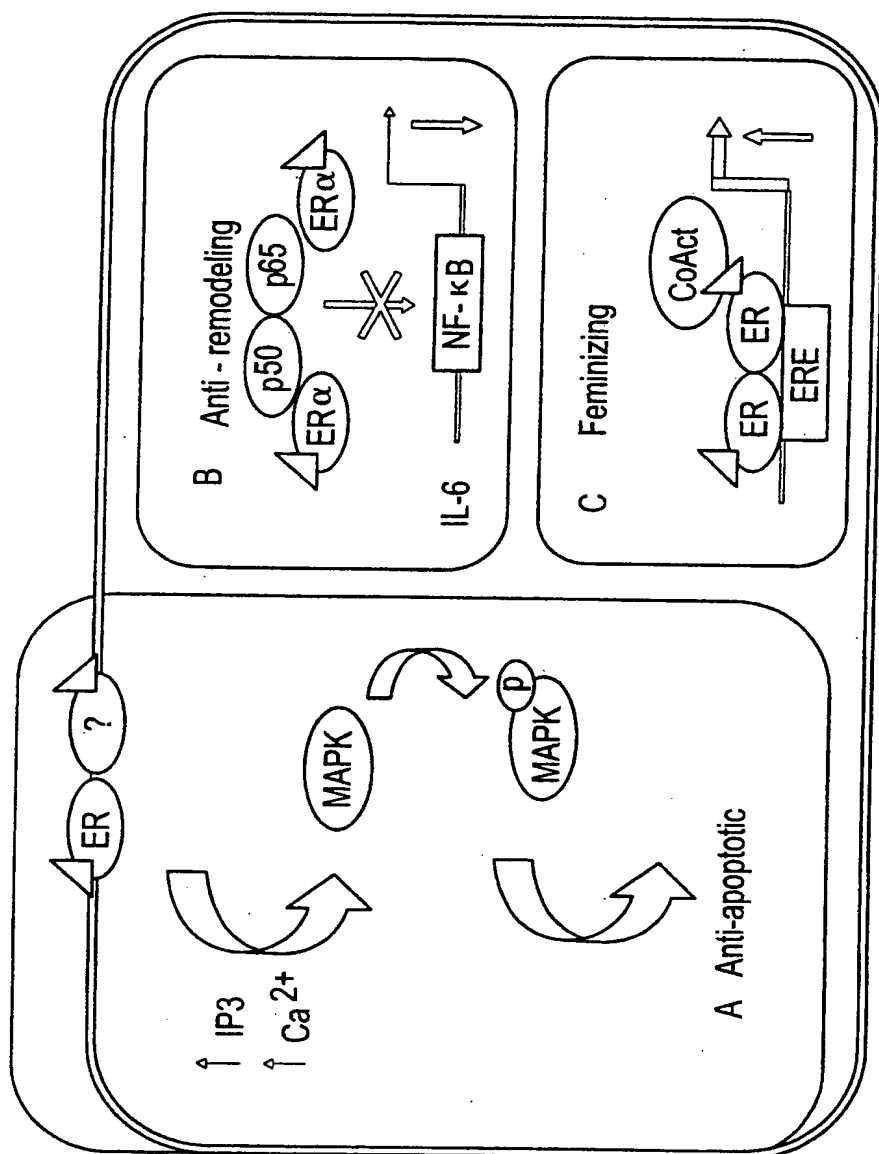
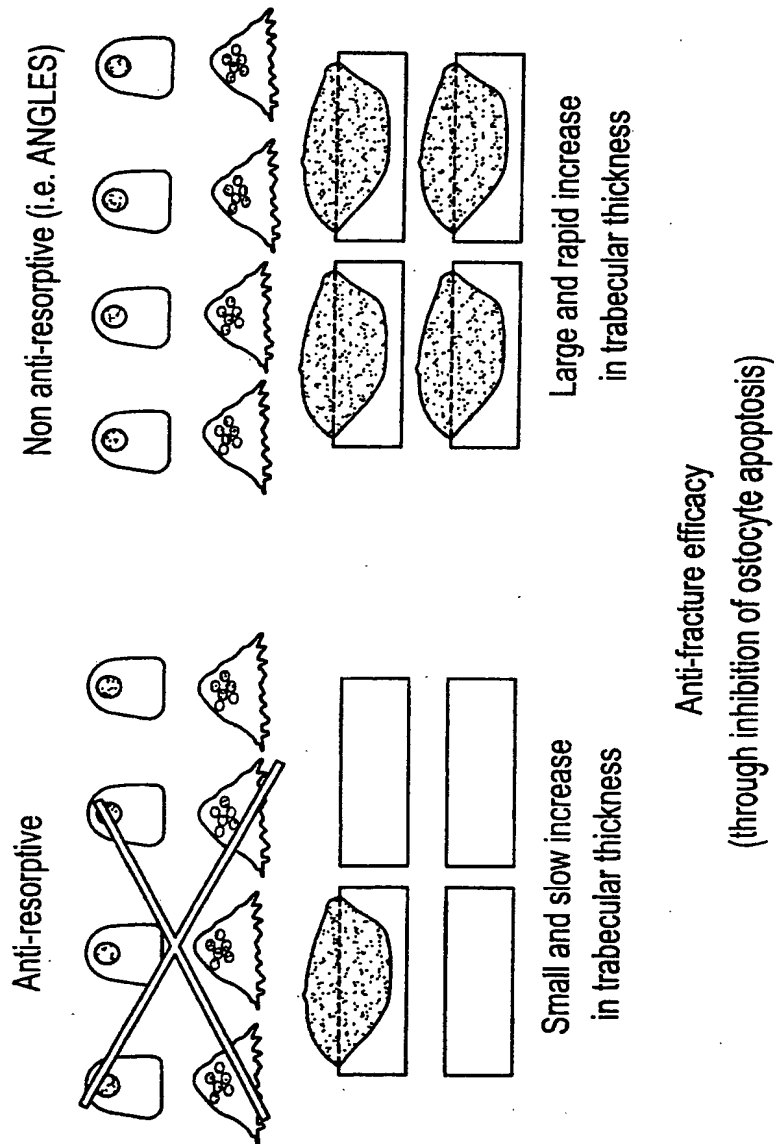


FIG. 14



Formation occurs only on sites of previous osteoclastic bone resorption.

FIG. 15



Implications of the effects of anti-resorptive vs. non anti-resorptive agents on apoptosis

FIG. 16A

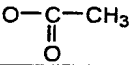

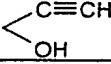
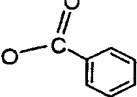
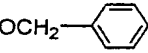
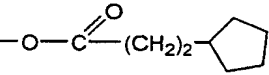
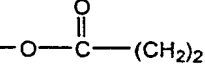
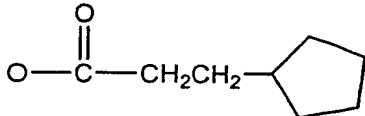
R ₁ AND/OR R ₂ SUBSTITUTIONS	
NAME	STRUCTURE
HYDROXYL	-OH
METHYL	-CH ₃
METHYL ESTER	-OCH ₃
ACETATE	
ETHYL ETHER	O-CH ₂ -CH ₃
3, 3, (OR 17, 17) DIMETHYL KETAL	
ETHYNYL-α	
BENZOATE	
BENZYL ETHER	
GLUCURONIDE	C ₆ H ₈ O ₆
SULFATE, SODIUM SALT	OSO ₃ Na
OXIDE	=O
VALERATE	-C ₅ H ₈ O
CYCLOPENTYLPROPIONATE	
PROPIONATE	
HEMISUCCINATE	-C ₄ H ₄ O ₃
PALMITATE	-C ₁₆ H ₃₂ O ₂

FIG. 16B

R ₁ AND/OR R ₂ SUBSTITUTIONS	
NAME	STRUCTURE
SODIUM PHOSPHATE	-O-PO ₃ Na ₂
ENANTHATE	-C ₇ H ₁₂ O
GLUCURONIDE, SODIUM SALT	-C ₆ H ₈ O ₆ Na
STEARATE	-C ₁₈ H ₃₄ O
TRIETHYL AMMONIUM SALT	-N-(C ₂ H ₅) ₃
CYPIONATE	 <chem>CCCCC(CC)C(=O)OC</chem>

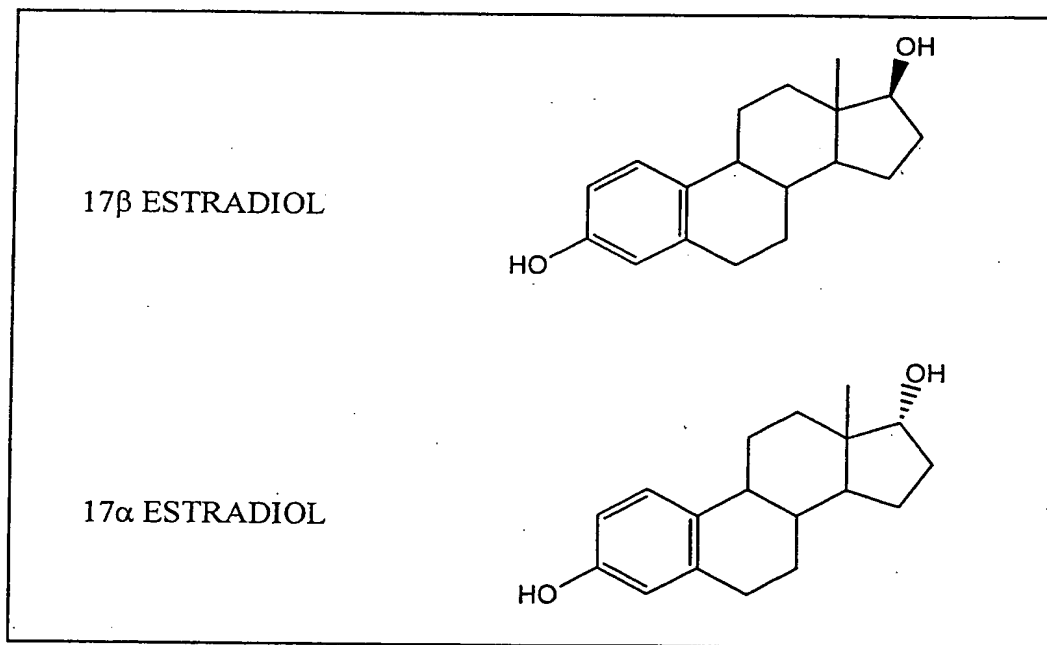


FIG. 17

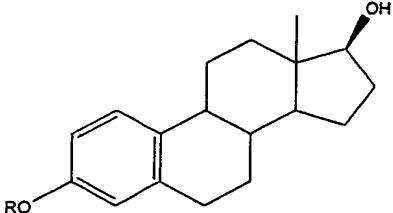
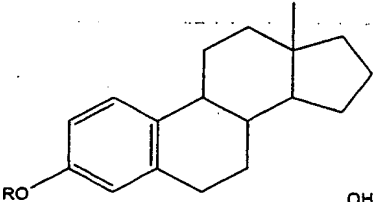
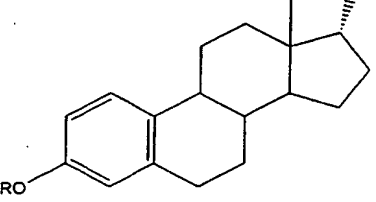
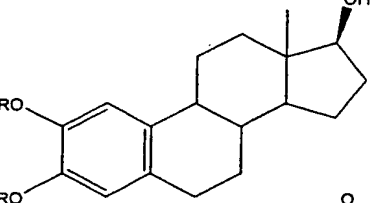
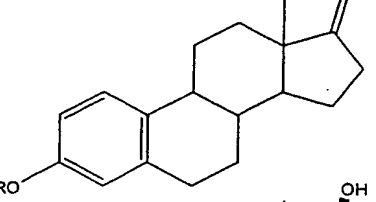
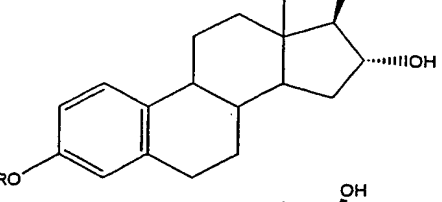
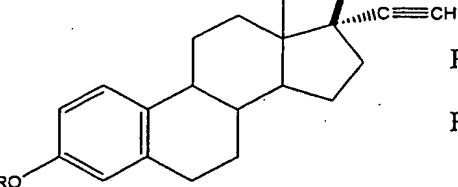
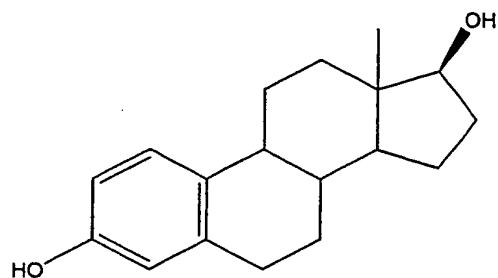
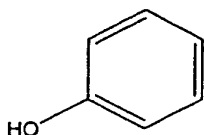
	Name	
	R=H	3,17β-Estradiol
	R=CH ₃	3,17β-Estradiol 3-O-ME
	R=H	Estratriene-3-ol
	R=H	3,17α-Estradiol
	R=CH ₃ CO	3,17α-Estradiol 3-acetate
	R=H	2-Hydroxy-17β-estradiol
	R=CH ₃	17β-Estradiol 2,3-O-ME
	R=H	Estrone
	R=CH ₃	Estrone 3-O-ME
	R=H	Estriol
	R=CH ₃	Estriol 3-O-ME
	R=H	Ethynyl Estradiol
	R=CH ₃	Mestranol

FIG. 18

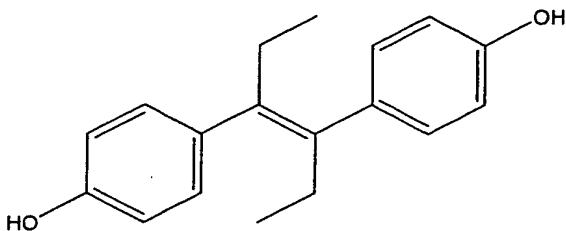


Name

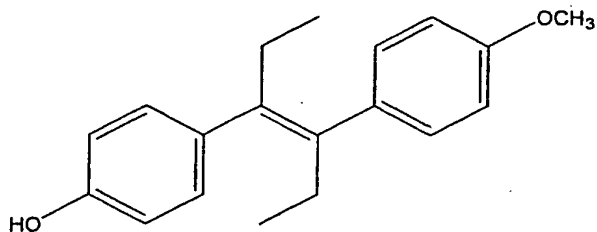
3,17β-Estradiol



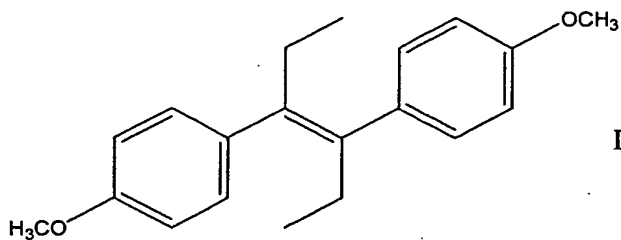
Phenol



Diethylstilbesterol

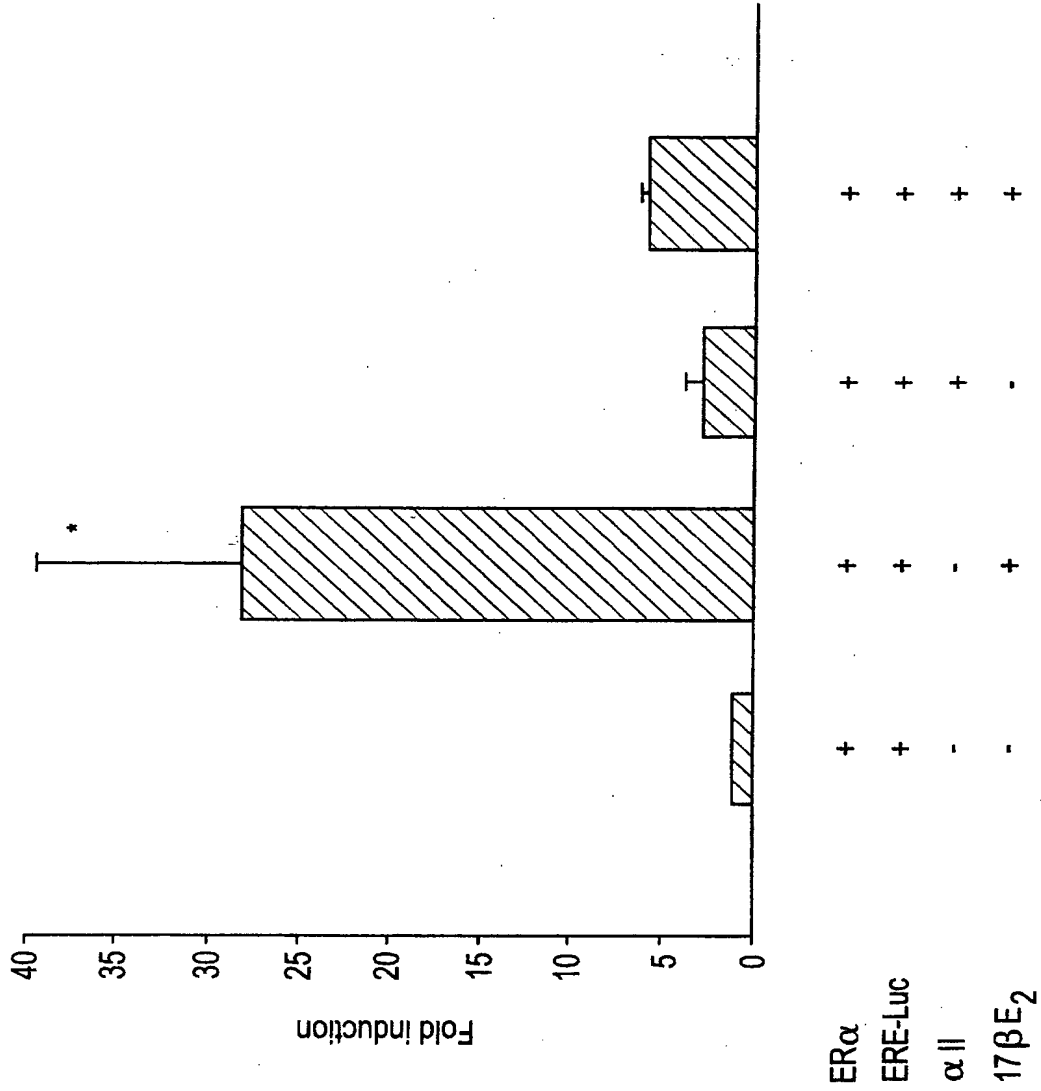


Diethylstilbesterol-mono-O-ME



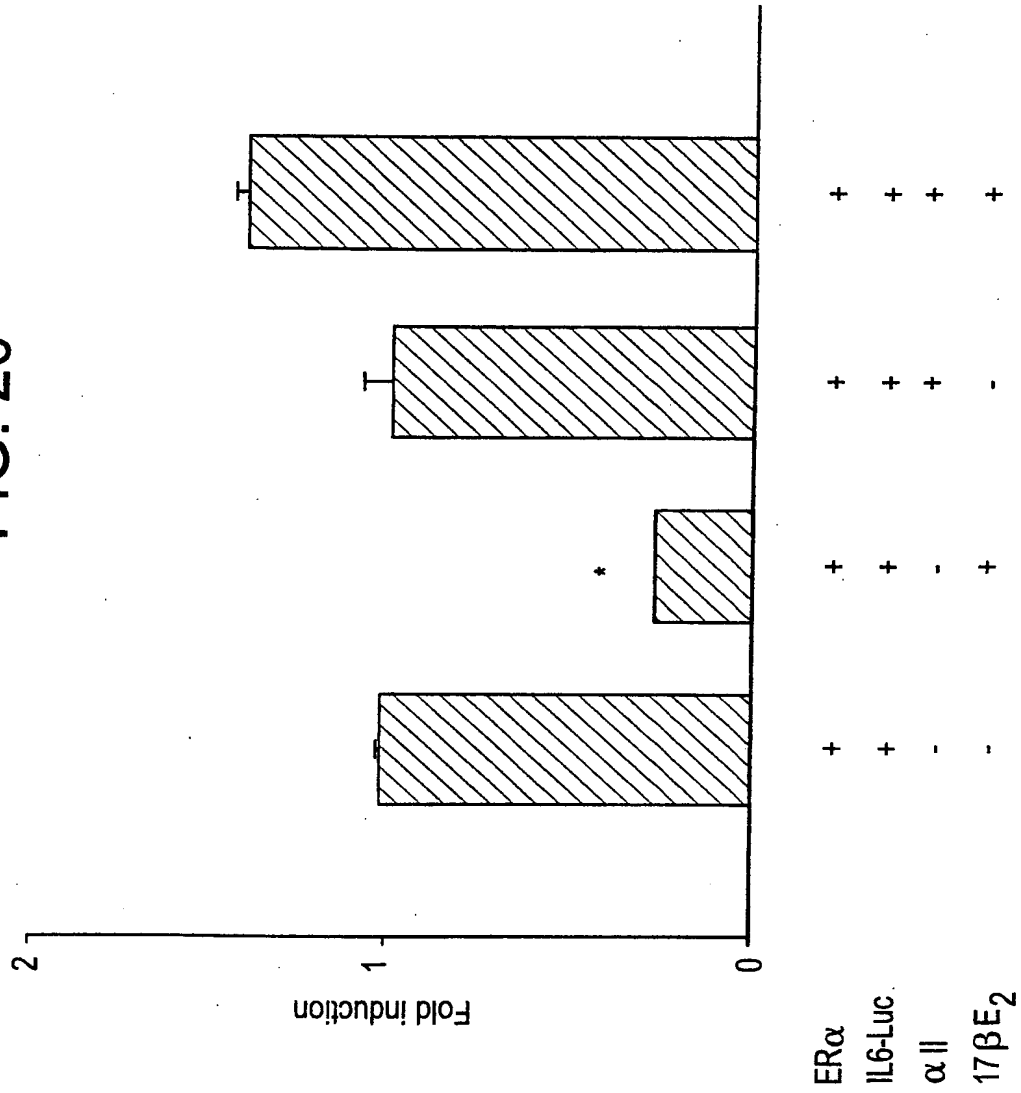
Diethylstilbesterol-di-O-ME

FIG. 19



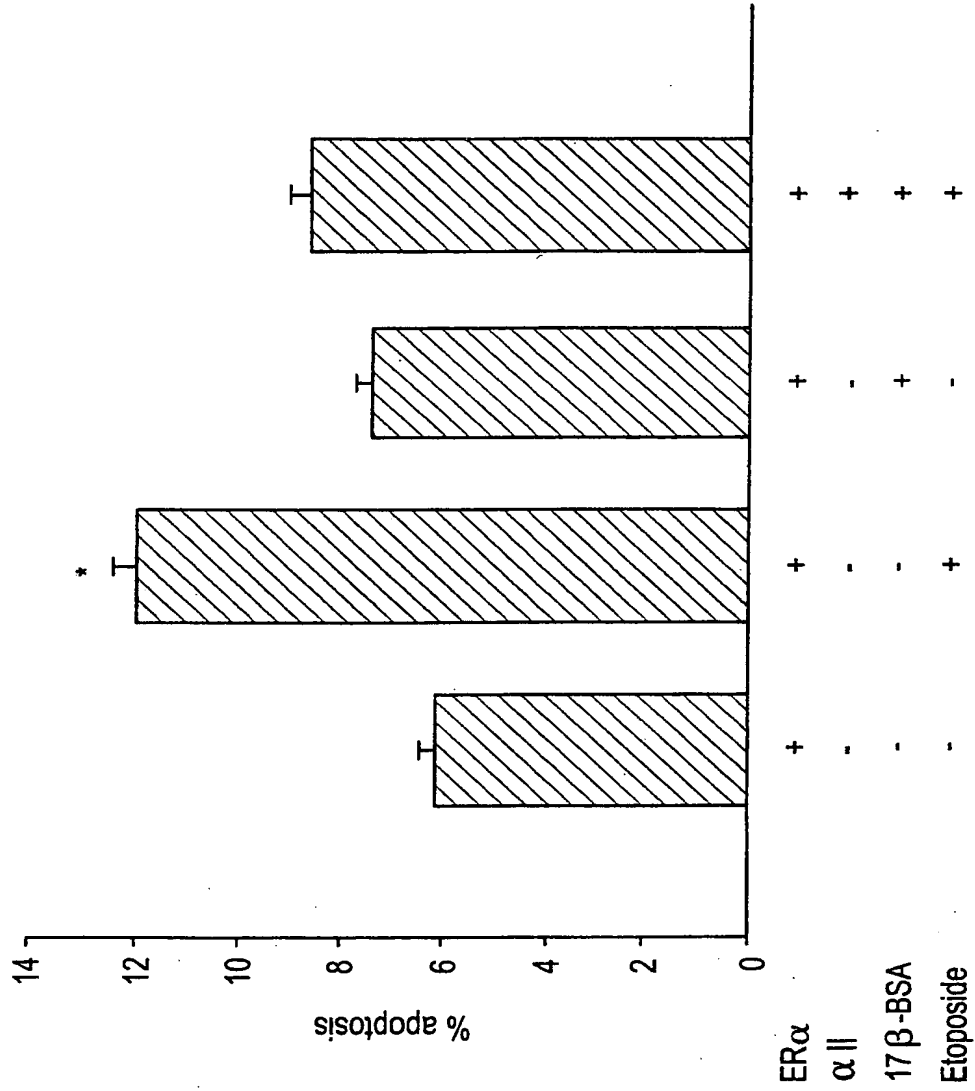
Effect of the all peptide on the 17βE₂-induced ERE activity in 293 cells

FIG. 20



Effect of the all peptide on the 17βE₂-induced inhibition of IL-6 activity in 293 cells

FIG. 21



Effect of the all peptide on the Etoposide-induced apoptosis of the 17b-BSA-activated 293 cells